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Discovery and SAR of Diarylsulfide Cyclopropylamide LFA-1/ICAM-1 Interaction Antagonists

James T. Link,* Bryan Sorensen, Gang Liu, Zhonghua Pei, Edward B. Reilly, Sandra Leitza and Greg Okasinski

Metabolic Disease Research, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA

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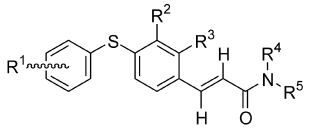
Abstract—Diarylsulfide cyclopropylamides were synthesized and evaluated as LFA-1/ICAM-1 interaction antagonists. A substituent pattern was identified which maximized potency and minimized protein binding as exemplified by antagonist **30** ($IC_{50} = 5$ nM). © 2001 Elsevier Science Ltd. All rights reserved.

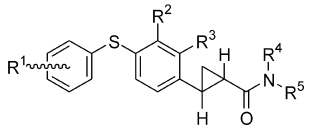
Cell adhesion is the process by which leukocytes are recruited to a site of injury.[1–3] The process is mediated by adhesion molecules on circulating leukocytes and on vascular endothelial cells. The interaction between leukocyte function-associated antigen-1 (LFA-1) on leukocytes and intercellular adhesion molecule (ICAM-1) on endothelial cells is critical to the adherence of leukocytes prior to extravasation from the vasculature into the surrounding tissue. Inhibition of the LFA-1/ICAM-1 interaction would presumably suppress this early step of the inflammatory response. An inhibitor could prove useful for the treatment or prophylaxis of inflammatory diseases, autoimmune diseases, tumor metastasis, allograft rejection, and reperfusion injury.

Early LFA-1/ICAM-1 interaction antagonists were mAb based due to the difficulty of identifying small molecule protein–protein interaction antagonists.[4–6] Recently, four different classes of small molecule antagonists have been identified,[7–10] including *trans*-cinnamide diarylsulfides **1** from our laboratories (Fig. 1).[11] Initially, we were concerned about the potential metabolic sensitivity of the diarylsulfide cinnamide antagonists and incubated one analogue with rat and human liver microsomes. This study indicated the cinnamide diarylsulfide was susceptable to cinnamide isomerization followed by degradation. Although it ultimately proved unneccessary to modify the cinnamide, a number of cinnamide replacements were surveyed including the diarylsulfide *trans*-cyclopropylamides **2**. Of the potential replacements surveyed, the cyclopropylamides were the most potent in vitro. In this paper, we present some of the *trans*-cyclopropylamide SARs and related observations which allowed us to advance our program.

The diarylsulfide cyclopropylamides were prepared as outlined in Scheme 1. Benzenethiols, such as 2-bromobenzenethiol **3**, were added to 4-halo- or 4-trifloxybenzaldehydes, like **5**,[12] under basic conditions to provide diaryl sulfide aldehydes **6**. Conversion of the aldehydes to the cinnamic esters **7** was accomplished by treatment with (ethoxycarbonylmethyl)triphenyl-phosphonium chloride. Cyclopropane formation utilizing trimethylsulfoxonium iodide yielded the *trans*-cyclopropyl esters **8**. Hydrolysis (**8**–**9**) and amide formation protocols, illustrated with amines like 1-(3-aminopro-



1 *trans*-cinnamide



2 *trans*-cyclopropylamide

Figure 1.

*Corresponding author. Fax: +1-847-938-1674; e-mail: james.link@abbott.com

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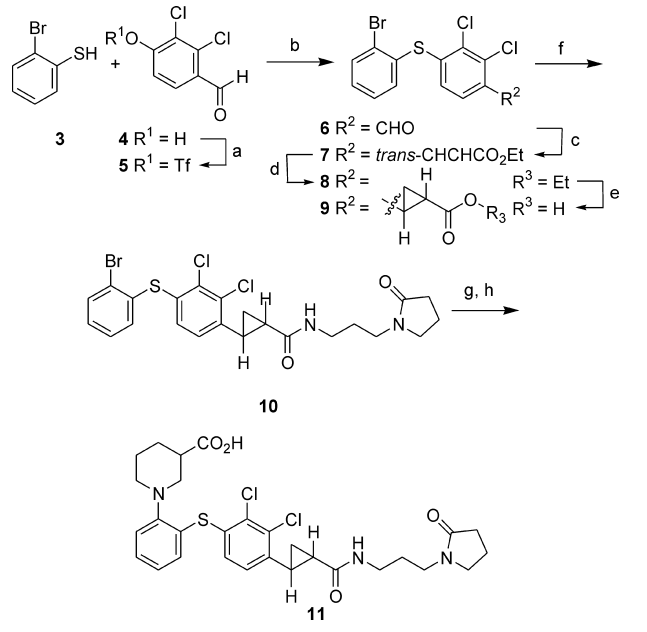
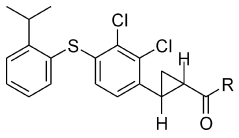


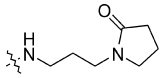
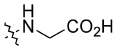
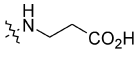
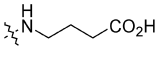
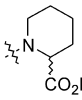
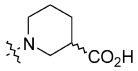
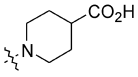
Scheme 1. (a) Tf_2O, pyr, 0 °C→rt, 1 h; (b) *i*-Pr_2NEt, CH_3CN, 25 min, 74%; (c) $EtO_2CCH_2PPh_3Cl$, NaH, THF, 2 h, 85%; (d) Me_3SOI, NaH, DMSO, 12 h, 65%; (e) NaOH, THF, EtOH, H_2O, 98%; (f) 1-(3-aminopropyl)-2-pyrrolidinone, EDAC, HOBt, DMF, rt, 95%; (g) ethyl nipecotate, $Pd_2(dba)_3$, (±)-BINAP, NaO*t*-Bu, tol, 80 °C, 12 h, 84%; (h) NaOH, THF, EtOH, H_2O, 97%.

pyl)-2-pyrrolidinone, yielded diarylsulfide cyclopropyl cinnamides **10**. Further modification using Buchwald/Hartwig aryl halide amine coupling protocols led to piperidine substituted analogues like **11**.[13] Resolution of intermediates by preparative HPLC on chiral columns was accomplished at either the cyclopropyl ester or amide stages depending upon the ease of the individual separation. Parallel synthesis allowed for rapid optimization of both the amide and piperidine regions of these analogues.

Like the cinnamides **1**,[11] diarylsulfide cyclopropamides **2** are most potent when R^1 is in the *ortho* or *meta* position (relative to the arylthio group) and R^2 and R^3 are small and electron withdrawing (Fig. 1). One potent series is obtained when R^1 is isopropyl and R^2 and R^3 are Cl (Table 1). Variation of the amide portion of the molecule revealed that a number of different groups (R^4 and R^5) are tolerated. The preferred substitutent at this position is 1-(3-aminopropyl)-2-pyrrolidinone as illustrated by **12a**, which gave an IC_{50} of 7 nM in an LFA-1/ICAM-1 binding assay.[14] Each *trans*-cyclopropylamide enantiomer of the diaryl sulfide core was evaluated independently. One cyclopropane antipode was consistantly more potent than the other (column 3 vs column 6). The more potent antipode was evaluated more thoroughly and an IC_{70} and IC_{80} were determined.

Table 1. Diarylsulfide cyclopropylamide SAR **12**–**18**



Compound	R	No serum IC_{50} (nM)[a,b]	No serum IC_{70} (nM)[a,b]	No serum IC_{80} (nM)[a,b]	Enantiomer no serum IC_{50} (nM)[a,c]
12a,b		7	40	63	6
13a,b		12	50	95	33
14a,b		8	26	54	34
15a,b		7	16	9	26
16a,b[d]		13	55	110	33
17a,b[d]		6	13	25	17
18a,b		9	24	54	21

[a]Values are means of two experiments.
[b]*trans*-Cyclopropylamide antipode **a**.
[c]*trans*-Cyclopropylamide antipode **b**.
[d]1:1 mixture of diastereomers.

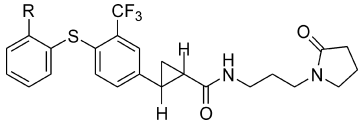
In order to ensure the agents had appropriate water solubility for in vivo utility it was necessary that they contain an acid. A number of amino acids were surveyed (**13**–**18**) and both straight chain and cyclic amino acids were tolerated.

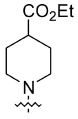
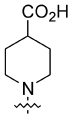
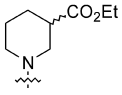
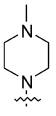
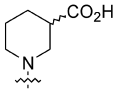
A cell based adhesion assay, which measured the ability of the antagonists to block the adherence of LFA-1 expressing JY-8 cells to immobilized ICAM-1, was used to confirm functional activity in vitro.[14] Among the compounds in Table 1, β-amino acid **14a** was the most potent antagonist in this assay giving an IC_{50} value of 150 nM ($n = 2$).

Examination of the potency of **12**–**18** in the presence of plasma protein yielded much higher IC_{50}'s. For example, cyclopropylamide **16a** (which was the least affected member of this set of compounds) gave an IC_{50} with no serum of 13 nM and an IC_{50} in the presence of 50% FBS of 1.48 μM. In the JY-8/ICAM-1 cellular adhesion assay with 50% serum, the analogues **12**–**18** were inactive at concentrations of 4 μM. In response to this protein binding problem we sought to alter the polarity of each region of the antagonist.

One region where we found substituent polarity could be varied was in the R^1 position. Racemic *trans*-cyclo-

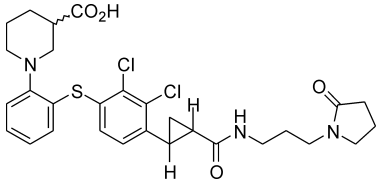
Table 2. Diarylsulfide cyclopropylamide SAR **19**–**26**



Compound	R	No serum IC_{50}, nM[a]	Compound	R	No serum IC_{50}, nM[a]
19		32	**23**		115
20		40	**24**		620
21		15	**25**[b]		100
22		200	**26**[b]		50

[a]Values are means of two experiments.
[b]Mixture of diastereomers.

Table 3. Diarylsulfide cyclopropylamide SAR **16a** and **27**–**30**



	No serum			50% FBS		
Compound	IC_{50} (nM)[a]	IC_{70} (nM)[a]	IC_{80} (nM)[a]	IC_{50} (nM)	IC_{70} (nM)	IC_{80} (nM)
16a	13	55	110	1480	3520	8820
27[b]	15	26	40	33	120	210
28[b]	10	21	26	29	116	199
29[c]	31	97	142	640	2100	3200
30[c]	5	10	19	27	89	133

[a]Values are means of two experiments.
[b]Mixture of two *trans*-cyclopropylamide diastereomers differing in the configuration of the 3-carboxypiperidine stereocenter of the antagonists.
[c]Single diastereomer derived from **28**.

propylamides **19**–**26** were synthesized in parallel. Pyrrolidinyl and piperidinyl analogues (**19** and **20**, respectively) had reasonable potency, which was improved upon by morpholine analogue **21**, which gave an IC_{50} value of 15 nM in an LFA/ICAM assay (Table 2). Placement of more polar substituents chosen to potentially improve water solubility at various positions around the ring resulted in less potent analogues (**22**–**25**). However, the racemic mixture of diastereomers **26** gave an IC_{50} quite close to that of the unsubstituted piperidine **20** indicating an acid could be placed at this position.

Moving to a more potent series and varying R^1 substituents a group of potent antagonists with reduced protein binding affinity was identified. Palladium-mediated coupling of ethyl nipecotate and hydrolysis of each enantiomer of *trans*-cyclopropylamide **10** gave **27** and **28**, respectively, each as a 1:1 mixture of diastereomers. Both diastereomeric mixtures **27** and **28** show good activity in the presence of serum in the LFA-1/ICAM-1 binding assay and represent a significant improvement over the profile observed for **16a** (Table 3). Diastereomeric mixture **28** was separated into its components **29** and A-324920 (**30**). Carboxypiperidine A-324920 (**30**) represented an advance for our program identifying a substituent pattern which delivered potency while minimizing protein binding. In the absence of serum, A-324920 (**30**) gave an IC_{50} of 5 nM in the LFA-1/ICAM-1 binding assay while in the presence of 50% FBS an IC_{50} of 27 nM was obtained. The 5-fold reduction in potency was a vast improvement over previous analogues like **16a**, which showed a greater than two orders of magnitude reduction. In the JY-8/ICAM-1 cellular adhesion assay A-324920 (**30**) gave an IC_{70} of 9 nM in the absence of serum. In the presence of 50% serum an IC_{50} of 50 nM and an IC_{70} of 200 nM were measured.

The pharmacokinetics of **28** in rats at 5 mpk po was measured. Although the bioavailability (F = 27%), half life ($t_{1/2}$ = 1.2 h), and maximum observed concentration in the bloodstream (C_{max} = 0.35 μg/mL) were not optimal, the results suggested that further refinement of closely related structures could potentially yield a compound with more desireable properties.

Lead modification of *trans*-cinnamide **1** led to the identification of *trans*-cyclopropylamides **2**. SAR studies indicated that potent LFA-1/ICAM-1 *trans*-cyclopropylamides could be made. Further optimization identified analogues with improved protein binding profiles as exemplified by antagonist A-324920 (**30**) (IC_{50} = 5 nM).

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14. The procedures for the LFA-1/ICAM-1 binding assay, the ICAM-1/JY-8 cell adhesion assay, and pharmacokinetic analyses are reported in ref 11.